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TISSUE CULTURE VARIABILITY IN WHEAT GERM–PLASM: CALLUS INITIATION AND LONG-TERM PLANT REGENERATION AND MAINTENANCE

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Twenty genotypes of spring and winter wheat were studied for their potential use in tissue culture. Calli were initiated from mature embryos on Linsmaier and Skoog's [LS] basal medium containing 4.0 mg/l 2,4-dichlorophenoxyacetic acid [2,4-D], 2% sucrose and 1% agar. Cultures were maintained on the same LS medium with 2,4-D reduced to 2.0 mg/l. Shoots were initiated from embryogenic callus [*E. callus*] by reducing 2,4-D to 0.1 mg/l and adding 0.1 and 0.5 mg/l indoleacetic acid [IAA] and benzylaminopurine [BAP] respectively. Complete plants were regenerated by transferring the calli to 2,4-D-free medium. Significant genotypic variation was observed for callus induction frequency, *E. callus* formation and the potential for plant regeneration. Cultures of ten genotypes remained morphogenic for 210 to 270 days. Three genotypes gave *E. callus* upto 455 days. For the first time wheat genotypes have been identified which were still able to regenerated plants after 900 days in culture (PI-410626, PI-299828, PI-478016). Of the 20 genotypes, PI-478022 and Dayak yielded the highest amount of *E. callus*, while PI-478022 and PI-478016 gave the highest number of regenerants. A total of 597 plants were regenerated and seeds were obtained. The genotypic effect on callus induction, long-term maintenance and plant regeneration in wheat germplasm is discussed.

Key words:Germplasm, Mature embryo, Long-term, Regeneration, Maintenance.

Introduction

The technology of growing cereal plants from either somatic or haploid cells has provided exciting new potential for plant improvement. Currently, all major cereal crops including rice (*Oryza sativa*) [1,2], barley (*Hordeum vulgare* L.) [3] maize [*Zea mays* L.] [4], pearl millet [*Pennisetum americanum*] [5], sorghum [*Sorghum bicolor*, Moench] [6] and wheat [*Triticum aestivum* L.] [7-9] have been grown in cell culture programmes with various degrees of success.

Several reports are available in the literature on callus induction and plantlet regeneration from various tissues of different wheat genotype [7,9-16] but much less information is available on their potential for germplasm improvement.

The search for specific genotypes that are capable of morphogenetic callus production, high rates of plant regeneration and long-term maintenance is an important step towards the application of tissue culture techniques to agriculture. The objectives of this study were to determine the response of wheat germplasm to *in vitro* culture, to develop techniques to regenerate plants from callus cultures and to establish long-term regenerable cultures. The genotypic effects on callus induction, growth response, regeneration and long-term maintenance and regeneration potential of wheat germplasm are discussed.

Materials and Methods

Callus induction. Seeds of 20 wheat genotypes (Table 1) were obtained from the Small Grain Storage Labs; Beltsville, Maryland, USA.

Caryopses of 20 wheat genotypes were surface sterilized for 30 sec. in 70% ethanol, then washed vigorously (using magnetic stir bar) with a 20% chlorox (commercial bleach of 5.25% sodium hypochlorite) solution plus a drop of Tween-20 for 20 mins followed by stirring for 3 mins in 0.05 mercuric chloride solution. After six rinses, seeds were left to soak for 8-12 hrs in the seventh rinse of sterile distilled water. Mature embryos were dissected out and were planted in vials containing basal salts of Linsmaier and Skoog (LS) [17], 4 mg/l 2,4-dichlorophenoxyacetic acid [2,4-D], 2% sucrose solidified with 1% agar. The medium was adjusted to pH 5.5 and autoclaved for 15 mins. at 15 psi and 120°. After 30 days in culture the 2,4-D level was reduced to 2 mg/l. The cultures were transferred to fresh medium at 30 day intervals thereafter. Embryos which germinated were discarded. On an average, 100 embryos were used per genotype.

Cultures were maintained in an environmentally controlled room at 22 + 3° in continuous light of 2000 lux at shelf height provided by four General Electric wide-spectrum fluorescent bulbs.

Callus induction frequency for each genotype was recorded after 30 days in culture. For callus growth measurements, calli from 20 jars were weighed individually at each passage.

Plant regeneration. The calli were divided into approximately 5 mm pieces and placed on LS medium with 2,4-D levels reduced to 0.1 mg/l. This 2,4-D level promotes shoot development but partially inhibits root development. Calli that produced shoots were then transferred to large jars (40 mm in

diameter and 100 mm in depth) containing 30 ml of medium without 2,4-D, but supplemented with 0.1 mg/l indoleacetic acid [IAA] and 0.5 mg/l 6-benzylaminopurine [BAP]. After shoot and root development regenerated plants were washed and then transplanted in small trays containing fine vermiculite. The trays were irrigated with nutrient solution and were transferred into a small growth chamber where high humidity (>90%) was maintained. After 15 days in the growth chamber, the plants were gradually transplanted into standard greenhouse potting soil (Sunshine Mix of Fission Western Corporation) and transferred to the greenhouse. The winter type genotypes were vernalized for five to six weeks then transplanted into standard greenhouse potting soil and grown to maturity. Plant sterility was tested by comparison with normal plants derived from seed at the time of maturity. Most of the data were subjected to analysis of variance using SAS/STAT statistical procedures [18]. For separating the mean values of different parameters and of various genotypes, Duncan's multiple range test was used.

Results and Discussion

Callus induction. Callus induction frequency, plants obtained after 150 days in culture and length of time cultures were maintained is summarized in Table 1 for 20 wheat genotypes. Significant genotype differences were observed among the genotypes for all parameters recorded.

Frequency of callus induction varied widely among the genotypes examined. The percentage of mature embryos that developed calli ranged from 0 to 100% with an average of 77.3% (Table 1).

Fresh weight of embryogenic callus produced by all genotypes after 150 days in culture is illustrated in Fig. 1. Genotypes differed significantly in *E. callus* formation which ranged from 0 in PI-410535 and PI-270025 to 0.713 g in PI-478022. The genotypes Dayak, PI-478016, PAK-16172 and PAK-15885 yielded the greatest amount of *E. calli* (Fig. 1), while PI-478243, PI-38887 and PI-388206 were poor yielders.

Calli colour varied widely and ranged from white and friable to slightly yellow and compact creamy green. The desired type i.e. compact creamy green callus, was dominant in PI-478016, PI-299828, Shorawaki, Dayak, PI-478056 and PI-410626 and these genotypes produced more regenerants as compared to the other (Table 1).

Plant regeneration. Cultures of genotypes which developed calli and remained alive beyond 150 days were tested for regeneration capacity. Regeneration was maintained by transferring these cultures to fresh medium. Green embryogenic calli when excised and placed on regeneration medium developed shoots and roots. Plantlets establishment was possible by elimination of the 2,4-D from the regeneration medium. The

TABLE 1. FREQUENCY OF CALLUS INDUCTION AND PLANT REGENERATION OF 20 WHEAT GENOTYPES.

Genotype	No. of embryos inoculated	Callus induced % of embryos inoculated	Plants after 150 days	Days morphogenic maintained
PAK-15885	100	100a*	85	455
PI-478016	100	94a	38	900
PI-299828	100	54b	55	900
PAK-15869	100	100a	45	450
PAK-16171	100	100a	42	270
PI-270019	100	70a	21	260
PI-270025	100	00d	00	00
PI-478243	100	96a	15	210
PI-478022	100	100a	27	260
Shorawaki	100	89a	30	240
PI-388187	100	78a	17	230
PI-410535	100	00d	00	00
PAK-16172	100	100a	40	250
PAK-16187	100	96a	40	270
PI-388221	100	76a	10	260
Dayak	100	100a	74	455
PI-388206	100	39c	18	720
PI-478111	100	98a	10	605
PI-410626	100	61b	20	900
PI-478056	100	63b	10	750
		Ave/ 77.3	Total 597	

* Means followed by the same letter in a column do not differ significantly at 5% level according to Duncan's multiple range test.

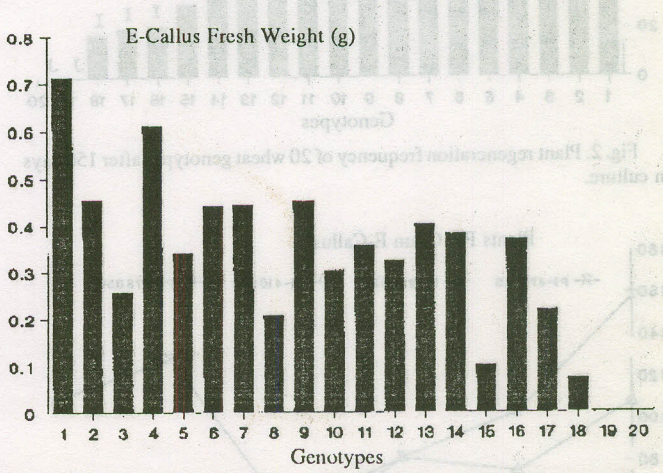

Fig. 1. Grams fresh weight of *E-callus* produced from mature embryo explant source of 20 wheat genotypes after five passages in culture.

1. PI-478022	2. PI-478016	3. PI-299828
4. Dayak	5. PAK-15869	6. PAK-15885
7. PAK-16187	8. PI-478243	9. PAK-16172
10. PI-478111	11. PAK-16171	12. Shorawaki
13. PI-478056	14. PI-410626	15. PI-388187
16. PI-270019	17. PI-388221	18. PI-388206
19. PI-410535	20. PI-270025	

*Bars followed by the same letter do not differ significantly according to Duncan's Multiple Range Test.

number of plants per gram of *E. calli* was calculated by putting known amounts of the *E. calli* on the regeneration medium. Significant genotype variation was observed for regeneration ability. The number of plants per gram of *E. callus* ranged from 0 to 151. PI-478022 produced the highest number of plants (151) followed by PI-478016, PI-299828 and Dayak (Fig. 2).

About 600 plants were regenerated from callus cultures maintained for 150 days (Table 1). Regenerative ability of the genotypes was tested after every five passages on maintenance medium. Three genotypes (PI-478016 PI-478056 and PI-410626) continued to maintain plant regeneration ability after 900 days in culture (Fig. 3). No regenerants were obtained from genotype PI-299828 after 750 days in culture. Regenerative ability in PI-478016 decreased after 150 days but improved and produced 128 plants per gram of *E. callus* after 750 days and 110 when regenerated after 900 days (Fig. 3).

The observed genotype variation in the regenerative ability of wheat germplasm is consistent with studies on corn [4],

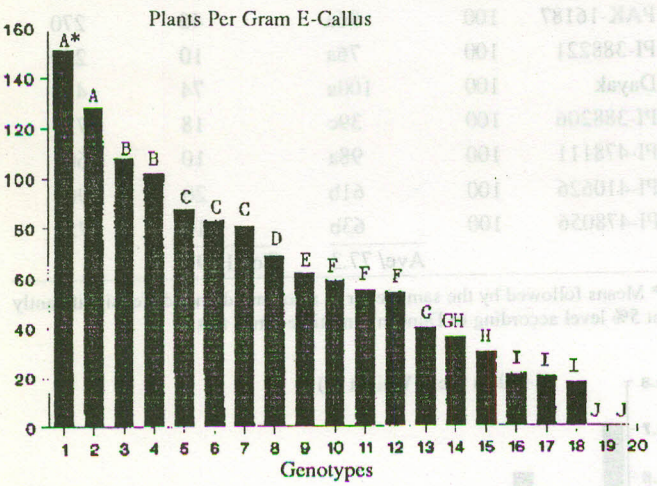

Fig. 2. Plant regeneration frequency of 20 wheat genotypes after 150 days in culture.

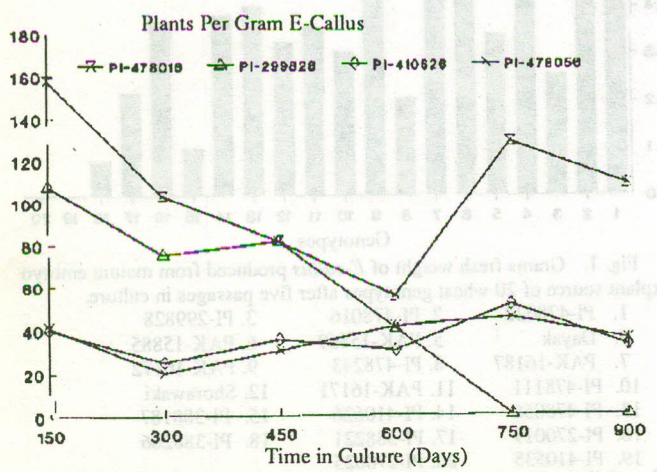

Fig. 3. Long-Term high frequency plant regeneration of four of 20 wheat genotypes screened.

wheat [7], rice [19], [20] and soybean [21]. The frequencies of regeneration obtained in the present study were higher than those reported in most studies using embryogenic callus in wheat. The reported studies used callus less than two month [24], three months [10, 11, 13], five months [22], eight months [23] and 12 months [25] in age for the regeneration in wheat. In the present study, plants were obtained from cultures maintained for more than 900 days and showed high regenerative ability (Fig. 3). In virtually all publications on regeneration, the reporting of the regeneration potential is not quantitative. Many authors claim high regeneration with some reporting percent of cultures with plants and few that actually reported the number of regenerants obtained.

In order to explore the true potential for the *in vitro* system in wheat, large number of genotypes, specifically the most valuable ones must be screened to identify those which allow callus growth during extended time periods with predictable and dependable plant regeneration. It was evident from these studies that substantial genotype variation exists for callus growth, regenerative ability and long-term maintenance of wheat germplasm. Cytological investigations and evaluations for morphological and physiological characteristics of the regenerants are underway.

Culture age has a great effect on the frequency of plant regeneration. MacKinnon [25] obtained high frequency long-term (ca. 12 months) regeneration in wheat but was not able to maintain and regenerate the cultures longer than 12 months. The search for such genotypes that are capable of morphogenetic callus production and high rates of plantlet regeneration is an important step in the application of tissue culture to agriculture. These genotypes should be a valuable source of material for studies such as selection of somaclonal variants adopted to environmental stresses.

Conclusion

In conclusion, results from these studies indicated that this wheat germplasm should be very suitable for in vitro techniques. In particular, PI-478016, PI-410626 and PI-478056 exhibited characteristics that should enable us to exploit cell selection techniques for germplasm improvement. Moreover, genotypes of high plant regeneration and long-term maintenance competence described in this report are useful for analysis of physiological and genetical factors controlling organogenesis in wheat.

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